Mouse HBsAb ELISA Kit

Catalog No.: abx054379

Range: Qualitative

Sensitivity: Qualitative

Storage: Store at 2-8°C for 6 months.

Application: For qualitative detection of HBsAb in Mouse Serum, Plasma, Tissue Homogenates and other biological fluids.

Introduction: Hepatitis B virus, abbreviated HBV, is a species of the genus Orthohepadnavirus, which is likewise a part of the Hepadnaviridae family of viruses. This virus causes the disease hepatitis B. In addition to causing hepatitis, infection with HBV can lead to cirrhosis and hepatocellular carcinoma. It has also been suggested that it may increase the risk of pancreatic cancer. The virus is divided into four major serotypes (adr, adw, ayr, ayw) based on antigenic epitopes present on its envelope proteins. These serotypes are based on a common determinant (a) and two mutually exclusive determinant pairs (d/y and w/r). The viral strains have also been divided into ten genotypes (A–J) and forty subgenotypes according to overall nucleotide sequence variation of the genome. The genotypes have a distinct geographical distribution and are used in tracing the evolution and transmission of the virus. Differences between genotypes affect the disease severity, course and likelihood of complications, and response to treatment and possibly vaccination.

Principle of the Assay
A 96 well plate has been pre-coated with an antigen specific to HBsAb. Controls or test samples are added to the appropriate wells and incubated. Free components are washed away with wash buffer. HRP conjugated detection reagent is used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue colour product that changes to yellow after adding acidic stop solution. The intensity of the color yellow is proportional to the HBsAb amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450nm in a microplate reader, and the presence of HBsAb can be determined.

Kit components
1. One pre-coated 96 well plate
2. Positive Control: 0.5ml
3. Negative Control: 0.5ml
4. Wash buffer (30×): 20 ml. Dilution: 1:30
5. Sample diluent buffer: 6 ml
6. HRP Conjugate Reagent (RTU): 6 ml
7. Stop solution: 6 ml
8. TMB substrate A: 6 ml
9. TMB substrate B: 6 ml
10. Plate sealer: 2
11. Hermetic bag: 1

Material Required But Not Provided
1. 37°C incubator
2. Microplate reader (wavelength: 450nm)
3. Precision pipette and disposable pipette tips
4. Automated plate washer
5. ELISA shaker
6. 1.5ml tubes to prepare standard/sample dilutions.
7. Plate cover
8. Absorbent filter papers
9. 100 ml and 1 L volume graduated cylinders

Protocol

A. Preparation of sample and reagents

1. Sample

Isolate the test samples soon after collecting and analyze immediately (within 2 hours) or aliquot and store at -20°C or -80°C for long term storage. Avoid multiple freeze-thaw cycles.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum at room temperature (~2 hr) or overnight at 4°C. Centrifuge at approximately 1000 × g for 20 min. Analyze the serum immediately or aliquot and store at -20°C or -80°C.
- **Plasma:** Collect plasma using citrate or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store at -20°C. Avoid hemolysis and high cholesterol samples.
- **Tissue homogenates:** The preparation of tissue homogenates will vary depending upon tissue type – this is just an example. Rinse tissues with ice-cold PBS to remove the excess of blood. Weigh before homogenization. Finely mince tissues and homogenize with a tissue homogenizer on ice in PBS and sonicate the cell suspension. Centrifuge the homogenates at 5000 × g for 5 min and collect the supernatant. Assay immediately or aliquot and store at -20°C.
- **Other biological fluids:** Centrifuge at approximately 1000 × g for 20 min to remove precipitant. Analyze immediately or aliquot and store at -20°C or -80°C.

Note:

» Coagulate blood samples completely, centrifuge, and avoid hemolysis and precipitant.

» NaN3 cannot be used as test sample preservative, since it inhibits HRP.

2. Wash buffer

Dilute the concentrated Wash buffer 30-fold (1/30) with distilled water (i.e. add 20 ml of concentrated wash buffer into 580 ml of distilled water).

B. Assay Procedure

Equilibrate the kit components and samples to room temperature prior to use. It is recommended to plot a standard curve for each test.

1. Set positive/negative, test sample and control (zero) wells on the pre-coated plate respectively and record their positions.
2. Aliquot 50 µl of the negative and positive controls into the set wells. Leave one well as the control (zero) blank well.
3. Aliquot 50 µl of appropriately diluted sample into the test sample wells. Add the solution at the bottom without touching the side walls of the well. Shake the plate mildly to mix the contents.
4. Seal the plate with a cover and incubate at 37°C for 30 min.
5. Remove the cover and discard the plate contents by tapping the plate on absorbent filter papers or other absorbent material.
6. Wash the plate 5 times with wash buffer. Do not let the wells completely dry at any time.

Manual Washing: Discard the solution without touching the side walls. Tap the plate on absorbent paper or other absorbent material. Fill each well completely with wash buffer and vortex mildly on ELISA shaker for 2 min. Discard the contents and tap the plate on absorbent filter papers or other absorbent material. Repeat this procedure five times.

Automated Washing: Discard the solution in all wells and wash the plate five times with Wash buffer (overfilling the wells with the buffer). After the final wash invert the plate and tap on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 min.

8. Add 50 µl of HRP conjugate reagent into each well (except control well). Add the solution at the bottom of each well without touching the side wall.
9. Seal the plate with a cover and incubate at 37°C for 30 min.
10. Remove the cover and repeat the aspiration/wash process 5 times as explained in step 7.
11. Aliquot 50 µl of TMB Substrate A into each well and 50 µl of TMB Substrate B. Vortex the plate gently on an ELISA shaker for 30 seconds (Or shake gently by hand for 30 seconds). Cover the plate and incubate at 37°C for 15 min. Avoid exposure to light.
12. Add 50 µl of Stop solution into each well. The color should change to yellow. Gently tap the plate to ensure thorough mixing.
13. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450nm immediately.

C. Analysis

1. Test effectiveness: the average value of positive control ≥ 1.00; the average value of negative control ≤0.10.
2. The critical value (CUT OFF) calculation: critical value = the average value of negative control + 0.15
3. Negative Result: if the OD value < CUT OFF, the sample is HBsAb negative.
4. Positive Result: if the OD value ≥ CUT OFF, the sample is HBsAb positive.

D. Precautions

1. Before using the kit, centrifuge the tubes to bring down the contents trapped in the lid.
2. Avoid foaming or bubbles when mixing or reconstituting components.
3. Wash buffer may crystallize and separate. If this happens, please heat the tube to dissolve.
4. It is recommended measuring each controls and samples in duplicate or triplicate.
5. Do NOT let the plate dry out completely as this will inactivate the biological material on the plate.
6. Ensure plates are properly sealed or covered during incubation steps.
7. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
8. Do not reuse pipette tips and tubes to avoid cross contamination.
9. Do not use expired components or components from a different kit.
10. Store the TMB substrate B in the dark and to avoid edge effect due to temperature difference during plate incubation it is recommended to equilibrate the TMB substrates for 30 min at room temperature prior to use. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.